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Research Article
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Synthesis, Structural Elucidation of Novel Thieno [2,3-d] Pyrimidine Core Unit Containing 1,2,4-Triazoles and Thiophenes as Potent Antimicrobial Activity

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Abstract

Several new thieno[2,3-d] Pyrimidine derivatives 3-Substituted phenyl-5-(thiophen-2-yl) thieno[3,2-e] [1,2,4] triazolo[4,3-c] pyrimidine 8(a-j), were synthesized starting from thieno[2,3-d] pyrimidine-2,4-diol (2). The characterization of the newly synthesized compounds was established by IR, ^{1}H NMR, ^{13}C NMR and Mass Spectral analysis. The final compounds were screened for their anti-bacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* from Gram positive group of bacteria and *Escherichia coli* and *Klebsiella pneumonia* from Gram negative group of bacteria and antifungal activity against *Candida albicans* and *Aspergillus flavus*. Anti-bacterial and anti-fungal activities were Evaluated and compared with the standard drugs. From anti-bacterial and anti-fungal activity screening results, it has been observed that compounds 8i, 8j, 8e possess good activity.

Keywords: Thieno [2, 3-d] pyrimidine; Antibacterial; Antifungal activity; Cyclisation; Suzuki cross coupling

Introduction

Pyrimidine has always been a unique interesting heterocyclic moiety for the medicinal chemists; an exhaustive research has been done on the pyrimidines that led to the discovery and introduction of several drugs into the market. From the standpoint of biological activity, fused hetero aromatic systems are often of much greater interest than the constituent monocyclic compounds. The appearance of qualitatively new properties of an annulated molecule, enlargement of the possibility of varying pharmacophore groups in different positions of the molecule and the ability of the latter to interact with a wider spectrum of receptors adopting various conformations are apparently of crucial importance. In addition, the structure of the molecule can be varied by annealing at different positions of individual Hetero cyclic fragments. Fused pyrimidines have also been attracted a considerable interest in medicinal chemistry research due to their versatility and a broad bioactive potential. Thieno pyrimidine is among those fused pyrimidines found to have a wide variety of pharmacological and biological applications. Since last four decades research has been focused on the design and synthesis of novel thienopyrimidines as medicinal agents, a large number of reports have been documented on thieno pyrimidines as they found to exhibit a variety of biological activities such as antimicrobial, anti-inflammatory, bronchodilatory activity, inhibition of Phospo diesterases, tyrosine kinase and VEGFR kinase. It is evident that purine as an endogeneous scaffold plays an important biochemical role in variety of regular physiological functions such as respiration, inflammation, cell proliferation and so forth. As a bio isoster to purines, thieno[2,3-d] pyrimidines were also found to exhibit numerous biological activities probably due to the interaction with various physiological elements. Thieno pyrimidine is a bi cyclic heterocyclic compound consists of a five membered thiophene ring is fused to a six membered hetero cylic ring with two nitrogen atoms. The fusion may occur in three different orientations that results in three important types of thieno pyrimidines namely; Thieno[2,3-d] Pyrimidine (a), thieno[3,2-d] Pyrimidine (b) and thieno[3,4-d] pyrimidine (c) (Figure 1). Most of the isomeric thienopyrimidines occur as colored amorphous form, some exists as crystalline form. Synthetic approaches for the construction of a number of thieno pyrimidines are well established. There exist three possible types of fusion of thiophene to pyrimidines ring results in corresponding isomeric thienopyrimidines namely; thieno [2,3-d] pyrimidines, thieno[3,4-d] pyrimidines and thieno[3,2-d] pyrimidines.

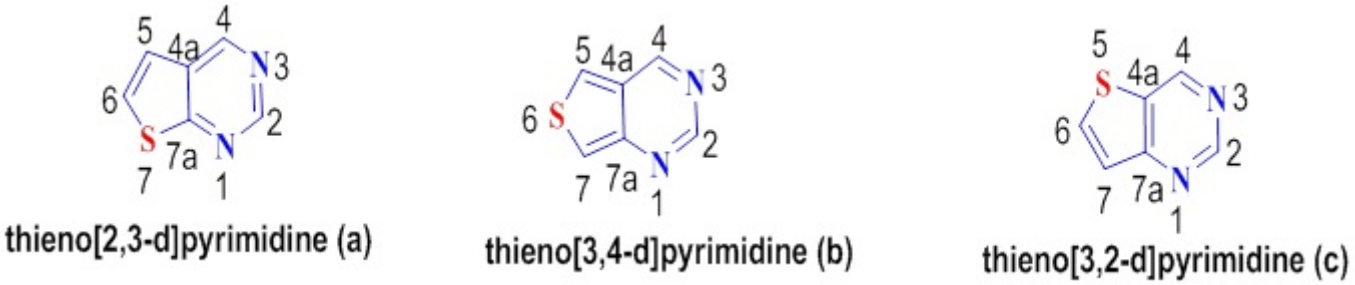


Figure 1: Three important types of Thieno pyrimidines.

Thieno pyrimidine derivatives, which are structure analogues of purines, have been focus of great interest because of their large range of pharmacological activities [1] as antibacterial, [2,3] antifungal [4], analgesic [5-7], antipyretic [8,9], anti-inflammatory [10], antihistaminic [11,12] anti-cancer [13-15] radioprotective [16,17]. Many thieno [2,3-d] pyrimidine derivatives were reported as phosphodiesterase inhibitors [18], also exhibited good H_1 receptor antagonistic activities [19], 4-amino derivatives showed insecticidal, pesticidal and acaricidal activities [20]. Numerous thieno[2,3-d] pyrimidines have been proved to use in case of cerebral ischemia, malaria, tuberculosis, Alzheimer's and Parkinson's diseases [21].

In this context, thienopyrimidine containing 1,2,4-triazole derivatives with similar structural qualities would be projected to result in newer molecular systems with increased efficacy. Definitely, 1,2,4-triazole template has been known to express considerable antimicrobial [22], antitubercular [23] and anticancer activities [24]. In continuation to extend our research [25,26] it was our thought of interest to design and synthesize thienopyrimidine 1,2,4 tri azole derivatives hoping to go a step forward in the field of antioxidant agents. Taking the above points in consideration, we have studied the antimicrobial action of the resultant thieno pyrimidine 1,2,4-triazole and Thiophene derivatives against wide range of different microorganisms.

This work aimed to synthesize some new thieno[2,3-d] pyrimdine derivatives starting with thieno[2,3-d] pyrimidine-2,4-diol and to evaluate their biological activities. Encouraged by the diverse biological activities of Thieno [2,3-d] pyrimidine Heterocyclic compounds, it was decided to prepare a new series of Thieno[2,3-d] pyrimidine Heterocyclic compounds. Literature survey revealed that incorporation of different groups in Thieno[2,3-d] pyrimidine Heterocyclic ring enhanced antibacterial and antifungal activity. In the present communication 2-chloro-4-hydrazinyl thieno[2,3-d] pyrimidine (4) was reacted with different substituted acids (5a-j) in $POCl_3$ at Reflux Temperature to form 1,2,4 tri azole Thieno Pyrimidine derivatives 6 (a-j), which were further reacted with thiophen-2-ylboronic acid (7) under Suzuki reaction conditions to get target compounds (8a-8j). The synthesis of the compounds as per the following Figure 2 given below. The synthetic route was depicted in Figure 2.

The structures of all synthesized compounds were assigned on the basis of IR, Mass, ^1H and ^{13}C NMR spectral data analysis. Further these compounds were subjected for antifungal and antibacterial activity.

Materials and Methods

In this Investigation chemicals were purchased from local dealer with S.D fine make was used. Chemicals were 99% pure; purity has been checked by thin layer chromatography and melting point. Conventional method has been used for synthesis of thieno[2,3-d] pyrimidine derivatives. Stirring and reflux method were used for synthesis of thieno[2,3-d] pyrimidine derivatives 8 (a-j) respectively. The synthetic route was depicted in Figure 2. The title compounds 8(a-j) were synthesized in five sequential steps using different reagents and reaction conditions (Figures 2 and 3), the 8(a-j) were obtained in moderate yields. The structure was established by spectral (IR, ^1H-NMR, ^{13}C-NMR and mass) and analytical data.

R=-Phenyl, -4 Methyl phenyl, -4 Methoxy phenyl, -4 tri fluoro methoxy phenyl, -4 Tri fluoro phenyl, -4 Chloro phenyl, -4 Bromo phenyl, -4 Nitro phenyl, -2 Indole, iso nicotinic acids

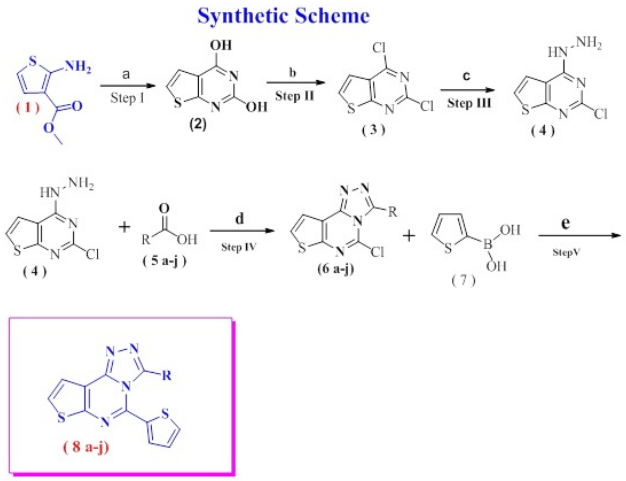


Figure 2: Synthetic path way for compounds 8a-8j. Synthetic path way of preparation of 1,2,4-triazole thienopyrimidine derivatives. Reagents and Reaction conditions: (a) Urea 200°C, 3 hrs (b) $POCl_3$, N-ethyl - N, N di isopropyl amine Reflux, 6 hrs (b) Hydrazine hydrate, Tri Ethyl Amine, Ethanol, 0°C-RT, 3 hrs. (d) $POCl_3$, Reflux, 6 hrs (e) 1,4-Di oxane, Na_2CO_3, Water, $Pd(PPh_3)4$, RT-110°C, 12 hrs.

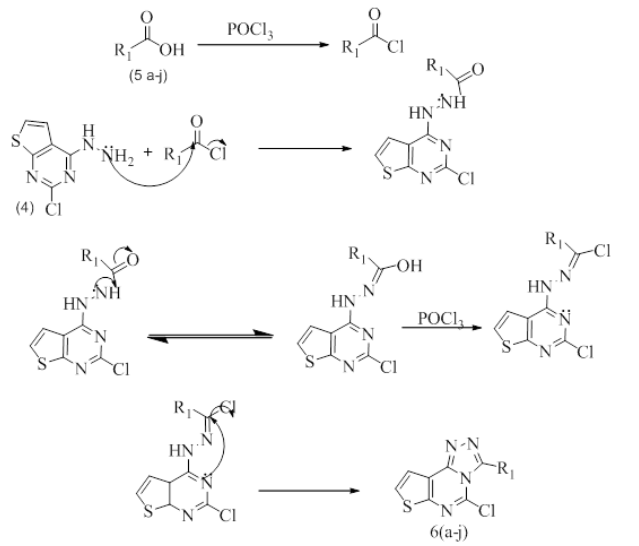


Figure 3: A plausible mechanism pathway for the formation of 1,2,4-triazole (6a-j).

Experimental Section

All reactions were carried out under argon in oven-dried glassware with magnetic stirring. Unless otherwise noted, all materials were obtained from commercial suppliers and were used without further purification. All solvents were reagent grade. THF was distilled from sodium benzo phenone ketyl and degassed thoroughly with dry argon directly before use. Unless otherwise noted, organic extracts were dried with anhydrous Na_2SO_4, filtered through a fitted glass funnel, and concentrated with a rotary evaporator (20-30 Torr). Flash chromatography was performed with silica gel (200-300 mesh) by using the mobile phase indicated. The NMR spectra were measured with a 400 MHz Bruker Avance spectrometer at 400.1 and 100.6 MHz, for ^1H for ^{13}C, respectively, in $CDCl_3$ solution with tetra methyl silane as internal standard. Chemical shifts are given in ppm (δ) and are referenced to the residual proton resonances of the solvents. Proton and carbon magnetic resonance spectra (^1H NMR and ^{13}C NMR) were recorded using tetra methyl silane (TMS) in the solvent of $CDCl_3$-d_1 or DMSO-d_6 as the internal standard (^1H NMR: TMS at 0.00 ppm, $CDCl_3$ at 7.26 ppm, DMSO at 2.50 ppm; ^{13}C NMR: $CDCl_3$ at 77.16 ppm, DMSO at 40.00 ppm).

General procedure for synthesis of thieno[2,3-d] pyrimidine-2,4-diol [compound (2)]

Methyl 2-aminothiophene-3-carboxylate (0.01 m.mol) and 0.05 m.mol of urea, were intimately mixed with each other, and the mixture was heated for two hours at 200°C. A clear, brown molten mass was formed which solidified upon standing; the solid product was dissolved in warm 1 N sodium hydroxide, and the resulting solution was decolorized with charcoal and then acidified with 2 N hydrochloric acid. The crystalline precipitate formed thereby was collected by vacuum filtration and recrystallized from Water, yielding 72% of thieno[2,3-d] pyrimidine-2,4-diol, MP 300°C above [27,28].

Yield: 90% (white color solid); IR (KBr, cm^{-1}): 3440 (-OH), 1160 (C-O-C Stretching), 3090 (Ar C-H), 1630 (Ar C=C Stretching).

^1H NMR (400 MHz; $CDCl_3$): δH 11.44 (S, ^1H, -OH), 9.18 (S, ^1H, -OH), 6.94 (d, 1H, J_{HH}=8.0 Hz, Ar-H),), 7.29 (d, J_{HH}=8.0 Hz, ^1H, Ar-H).

^{13}C NMR (100 MHz; $CDCl_3$): δC 128.92, 124.03, 128.11, 159.62, 151.67, 154.75.

LC-MS (70 eV): m/z=169 $(M+H)^+$.

General procedure for synthesis of 2,4-dichlorothieno[2,3-d] pyrimidine [compound (3)]

A mixture consisting of 8.4 gm (0.05 mol) of 2,4-di hydroxy-thieno[2,3-d] Pyrimidine (2) and 100 ml. of phosphorus oxychloride was refuxed for ten hours, whereby a clear solution was formed. Thereafter, the excess unre acted phosphorus oxy chloride was evaporated in vacuo, the residual oil was poured into ice water, and the aqueous mixture was extracted with chloroform [29]. The chloroform phase was isolated, washed with water until neutral, then dried over Sodium sulfate, the chloroform was evaporated in vacuo, and the solid residue was re-crystallized from ethanol. 7.6 gm. (75% of yield) of 2,4-dichloro thieno[2,3-d] pyrimidine, M.P. 161-162°C, were obtained.

IR (KBr, cm^{-1}): 740(-C-Cl), 3110(Ar C-H), 1660 (Ar C=C Stretching).

^1H NMR (400 MHz; $CDCl_3$): δH 6.98 (d, 1H, J_{HH}=7.0 Hz, Ar-H),), 7.39 (d, J_{HH}=7.0 Hz, 1H, Ar-H).

^{13}C NMR (100 MHz; $CDCl_3$): δC 126.92, 123.03, 126.11, 153.62, 161.67, 154.75.

LC-MS (70 eV): m/z=205$(M+H)^+$, 207(M+2), 209(M+4), 9:6:1 It indicates molecule contain two chlorine atoms.

General procedure for synthesis of 2-chloro-4-hydrazinylthieno [2,3-d] pyrimidine [compound (4)]

A mixture of 2,4-dichlorothieno[2,3-d] pyrimidine [compound (2)] (Compound 2) (0.1 mol) in methanol was taken and cooled to 0°C-5°C in an ice bath. Tri Ethyl amine (0.3 mol) was added to the cold reaction mixture and then hydrazine hydrate (0.15 mol) was added slowly at 5°C-10°C. The reaction mass was allowed to stir at room temperature for 3 hrs, after completion of starting compound, the excess amount of methanol and Tri Ethyl amine was removed under vacuum. The residue was washed with water, finally petroleum ether then they obtain solid was filtered and Dried under vacuum (Figures 4, 5 and 6).

Yield: 84% (pale brown color solid); m.p. 202-204°C.

IR (KBr, cm^{-1}): 760(-C-Cl), 3102(Ar C-H), 1650 (Ar C=C Stretching), 3340 (-N-H Stretching, two bands indicates 1°Amine).

^1H NMR (400 MHz; $CDCl_3$): δH 4.68 (s, 2H), 7.60 (S, 2H, Ar-H), 9.6 (1H, S).

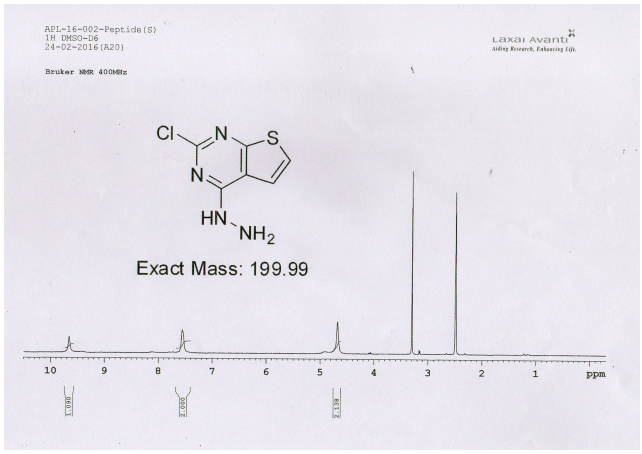


Figure 4: ^{1}H NMR of 2-chloro-4-hydrazinylthieno [2,3-d] pyrimidine.

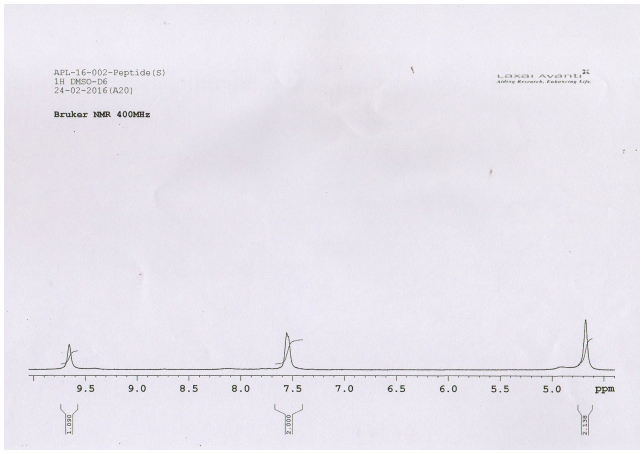


Figure 5: ^{13}C NMR (100 MHz; $CDCl_3$): δC 126.92, 123.03, 126.11, 173.62, 158.67, 154.25 MS (70 eV): m/z=201(M+H) +, 203(M+2), 3:1 It indicates molecule contain one chlorine atom.

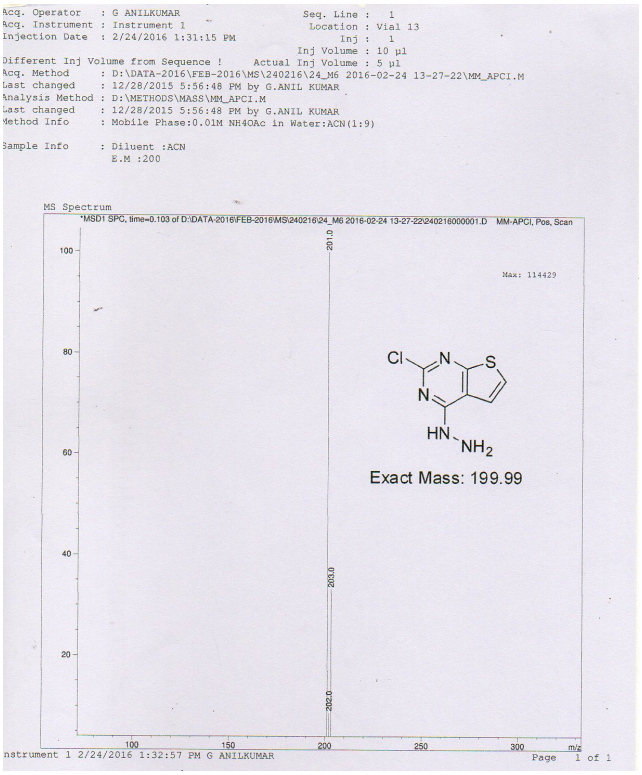


Figure 6: Exact mass after drying.

General procedure for synthesis of

Compound (4) (Figure 7) (0.1 m. mol) and substituted benzoic acids (or) Heterocyclic Acids (5a-j) (0.13 m.mol) were taken in $POCl_3$ [30] (5 ml) and heated to reflux for 6 hrs. The reaction mass was concentrated under reduced pressure and then quenched in ice water. The Solid obtained was filtered off, washed with water, dried and crystallized from methanol/Ethanol solvent.

The following compounds were synthesized using this method.

Yield: 84% (brown color solid); m.p. 212-214°C.

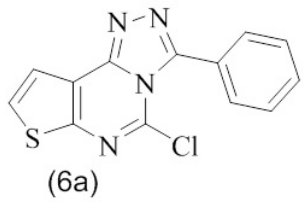
IR (KBr, cm^{-1}): 755(-C-Cl), 3100(Ar C-H), 1570 (Ar C=C Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 7.40-7.53 (m, 3H, Ar-H), 8.3(2H,t, Ar-H), 6.96 (1H, d, Ar-H), 7.3(1H,d, Ar-H).

^{13}C NMR (100 MHz; $CDCl_3$): δC 126.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131, 133.

LC-MS (70 eV): m/z=287$(M+H)^{+}$, 289(M+2), 3:1 It indicates molecule contain one chlorine atom (Figure 7).

5-chloro-3-phenylthieno[3,2-e][1,2,4] triazolo[4,3-c]pyrimidine (6a)



(6a)

Figure 7: 5-chloro-3-phenylthieno[3,2-e][1,2,4] triazolo[4,3-c]pyrimidine.

Yield: 81% (yellow color solid); m.p. 210-212°C.

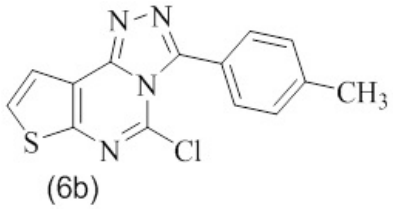
IR (KBr, cm^{-1}): 745(-C-Cl), 3110(Ar C-H), 1590 (Ar C=C Stretching), 2960(SP_3 C-H Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.96 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.3(1H,d,J_{HH}=7.0 Hz, Ar-H), 8.5(2H, d, J_{HH}=7.4 Hz, Ar-H), 7.3((2H, d, J_{HH}=7.4 Hz, Ar-H), 2.36(3H,S).

^{13}C NMR (100 MHz; $CDCl_3$): δC 126.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131, 21.3.

LC-MS (70 eV): m/z=301(M+H)+, 303(M+2), 3:1 It indicates molecule contain one chlorine atom (Figure 8).

5-chloro-3-p-tolylthieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (6b)



(6b)

Figure 8: 5-chloro-3-p-tolylthieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 84% (yellow color solid); m.p. 250-251°C.

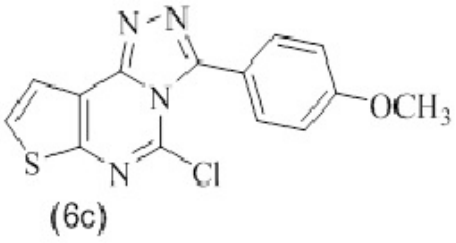
IR (KBr, cm^{-1}): 755(-C-Cl), 1155(C-O-C Stretching), 3095(Ar C-H), 1580 (Ar C=C Stretching), 2950(SP_3 C-H Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.96 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.3(1H,d, JHH=7.0 Hz, Ar-H), 7.95(2H, d, J_{HH}=7.8 Hz, Ar-H), 7.03((2H, d, J_{HH}=7.8 Hz, Ar-H), 3.86(3H,S).

^{13}C NMR (100 MHz; $CDCl_3$): δC 126.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131.

LC-MS (70 eV): m/z=317(M+H)$^+$, 318(M+2), 3:1 It indicates molecule contain one chlorine atom (Figure 9).

5-chloro-3-(4-methoxyphenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (6c):



(6c)

Figure 9: 5-chloro-3-(4-methoxyphenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 81% (Greenish yellow color solid); m.p. 190-191°C.

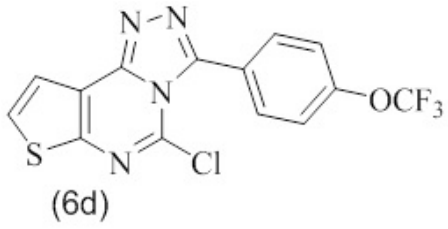
IR (KBr, cm^{-1}): 735(-C-Cl), 1340(C-F), 3110(Ar C-H), 1580 (Ar C=C Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.96 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.3(1H,d, JHH=7.0 Hz, Ar-H), 7.95(2H, d, J_{HH}=7.8 Hz, Ar-H), 7.03((2H, d, J_{HH}=7.8 Hz, Ar-H).

^{13}C NMR (100 MHz; $CDCl_3$): δC 126.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131.

LC-MS (70 eV): m/z=371(M+H)$^+$, 373(M+2), 3:1 It indicates molecule contain one chlorine atom (Figure 10).

5-chloro-3-(4-(trifluoromethoxy)phenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (6d)



(6d)

Figure 10: 5-chloro-3-(4-methoxyphenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 81% ((brown color solid); m.p. 216-218°C.

IR (KBr, cm^{-1}): 765(-C-Cl), 1340(C-F), 3110(Ar C-H), 1580 (Ar C=C Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.96 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.3(1H,d, JHH=7.0 Hz, Ar-H), 8.65(2H, d, J_{HH}=7.8 Hz, Ar-H), 7.73((2H, d, J_{HH}=7.8 Hz, Ar-H).

^{13}C NMR (100 MHz; $CDCl_3$): δC 126.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131, 124.

LC-MS (70 eV): m/z=355(M+H)$^+$, 357(M+2), 3:1 It indicates molecule contain one chlorine atom (Figure 11).

5-chloro-3-(4-(trifluoromethyl)phenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine (6e)

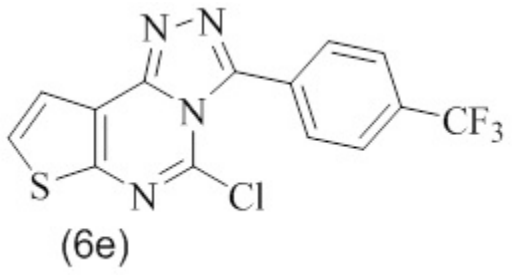


Figure 11: 5-chloro-3-(4-(trifluoromethyl)phenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine.

Yield: 81% (pale brown color solid); m.p. 186-188°C.

IR (KBr, cm^{-1}): 768(-C-Cl), 3120(Ar C-H), 1590 (Ar C=C Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.96 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.3(1H,d, JHH=7.0 Hz, Ar-H), 8.25(2H, d, J_{HH}=7.8 Hz, Ar-H), 7.53((2H, d, J_{HH}=7.8 Hz, Ar-H).

^{13}C NMR (100 MHz; $CDCl_3$): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131, 124.

LC-MS (70 eV): m/z=320(M+H)$^{+}$, 322(M+2), 324(M+4) 9:6:1 It indicates molecule contain two chlorine atoms (Figure 12).

5-chloro-3-(4-chlorophenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine (6f)

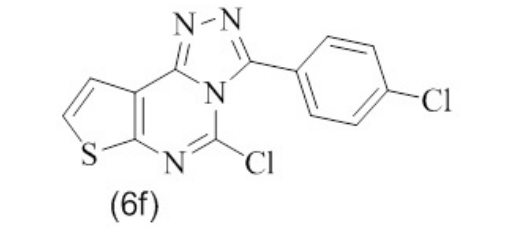


Figure 12: 5-chloro-3-(4-chlorophenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine.

Yield: 80% (pale yellow color solid); m.p. 218-220°C.

IR (KBr, cm^{-1}): 748(-C-Cl), 540(C-Br), 3120(Ar C-H), 1560 (Ar C=C Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.96 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.3(1H,d, J_{HH}=7.0 Hz, Ar-H), 7.65(2H, d, J_{HH}=7.8 Hz, Ar-H), 7.53((2H, d, J_{HH}=7.8 Hz, Ar-H).

^{13}C NMR (100 MHz; $CDCl_3$): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131, 124.

LC-MS (70 eV): m/z=364(M+H)$^{+}$, 366(M+2), 368(M+4) (Figure 13).

3-(4-bromophenyl)-5-chlorothieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (6g)

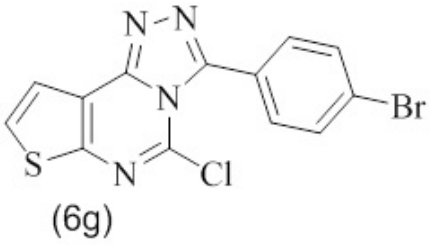


Figure 13: 3-(4-bromophenyl)-5-chlorothieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 80% (yellow color solid); m.p. 219-221°C;

IR (KBr, cm^{-1}): 768(-C-Cl), 1540 and 1350 (N-O Stretching in Nitro group), 3160(Ar C-H), 1590 (Ar C=C Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.76 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.2(1H,d, J_{HH}=7.0 Hz, Ar-H), 8.15(2H,d, J_{HH}=7.0 Hz, Ar-H), 8.53((2H, d, J_{HH}=7.0 Hz, Ar-H).

^{13}C NMR (100 MHz; $CDCl_3$): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 144, 128, 124, 150.

LC-MS (70 eV): m/z=330(M-H)+, 332(M+2) 3:1 It indicates molecule contain one chlorine atom (Figure 14).

5-chloro-3-(4-nitrophenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (6h)

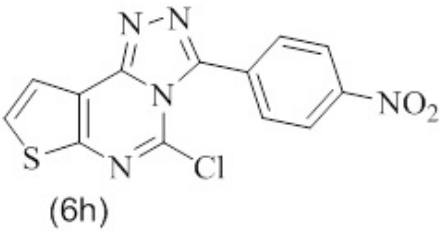


Figure 14: 5-chloro-3-(4-nitrophenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 70%s (greenish yellow color solid); m.p. 239-241°C.

IR (KBr, cm-^{1}): 768(-C-Cl), 3320 (N-H Stretching), 3120(Ar C-H), 1586 (Ar C=C Stretching).

^{1}H NMR (400 MHz; DMSO-d_6): δH 6.86 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.2(1H,d, J_{HH}=7.0 Hz, Ar-H), 6.8(1H,S), 6.9(2H, m, Ar-H), 7.53-7.6(2H, m, Ar-H).

^{13}C NMR (100 MHz; $CDCl_3$): δC 127.92, 123.03, 126.11, 153.62, 160.67, 143.25, 124, 100, 128, 120, 113.

LC-MS (70 eV): m/z=326(M+H)$^{+}$, 328(M+2) 3:1 It indicates molecule contain one chlorine atom (Figure 15).

5-chloro-3-(1H-indol-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine(6i)

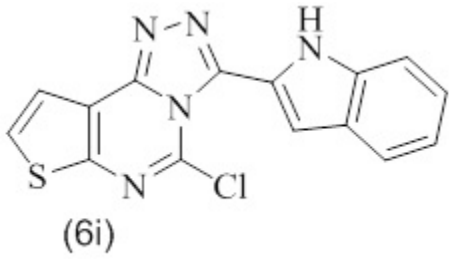


Figure 15: 5-chloro-3-(1H-indol-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 74% ((brown color solid); m.p. 240-242°C.

IR (KBr, cm^{-1}): 758(-C-Cl), 3110(Ar C-H), 1580 (Ar C=C Stretching).

^{1}H NMR (400 MHz; DMSO-d$_6$): δH 6.86 (1H, d,J_{HH}=7.0 Hz, Ar-H), 7.2(1H,d, J_{HH}=7.0 Hz, Ar-H), 8.5(1H,d, J_{HH}=7.4 Hz, Py. Ar-H), 7.6(1H, t, J_{HH}=7.4 Hz, Py. Ar-H), 8.7(1H, d, J_{HH}=7.4 Hz, Py. Ar-H), 9.3(1H, S, Py.Ar-H).

^{13}C NMR (100 MHz; CDCl$_3$): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 135, 124, 148, 155.

LC-MS (70 eV): m/z=288(M+H)$^+$, 290(M+2) 3:1 It indicates molecule contain one chlorine atom (Figure 16).

5-chloro-3-(pyridin-3-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (6j)

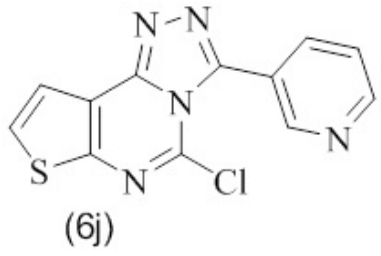


Figure 16: 5-chloro-3-(pyridin-3-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

General procedure for synthesis of

A mixture of compound-6a-6j (0.6 m.mol), compound-7 (0.9 m.mol), K_2CO_3 (3.2 m.mol) degassed with argon for 10 min. and Pd(PPh3)4 (0.0033 m.mol) in 5 ml 1,4 Di Oxane solvent at 100°C in a sealed tube for 16 hrs. Reaction progress was monitored by TLC. Then reaction mixture was diluted with water and Extracted with EtoAc, dried over Na_2SO_4 filtered and evaporated to dryness. The crude product was purified by Column Chromatography affording product 8(a)-8(j) (Figure 17).

Yield: 56% (Fine brown needles); m.p. 224-226°C.

IR (KBr, cm^{-1}): 655(-C-S-C Stretching), 3090(Ar C-H), 1686 (C=N Stretching), 1589 (Ar C=C Stretching).

^{1}H NMR (400 MHz; DMSO-d$_6$): δH 7.40-7.63 (m, 3H, Ar-H), 8.34(2H,t, Ar-H), 6.96 (1H, d, Ar-H), 7.3(1H,d, Ar-H), 7.8(1H,d), 7.2(1H,t), 8.3(1H,d).

^{13}C NMR (100 MHz; DMSO-d$_6$): δC 126.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131, 133.

LC-MS (70 eV): m/z: 333(M-H, 100%)+, 334(M+1, 18%), It indicates molecule contain 17 Carbon atoms (Figure 17).

3-phenyl-5-(thiophen-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine(8a)

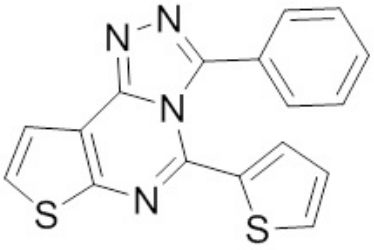


Figure 17: 3-phenyl-5-(thiophen-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 51% (Fine brown crystals); m.p. 118-120°C.

IR (KBr, cm^{-1}): 3110(Ar C-H), 1680 (Ar C=C Stretching), 2980(SP_3 C-H Stretching).

^{1}H NMR (400 MHz; DMSO-d$_6$): δH 6.96 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.3(1H,d, J_{HH}=7.0 Hz, Ar-H), 8.5(2H, d, J_{HH}=7.4 Hz, Ar-H), 7.3((2H, d, J_{HH}=7.4 Hz, Ar-H), 7.83(1H,d), 7.23(1H,t), 8.32(1H,d), 2.36(3H,S).

^{13}C NMR (100 MHz; DMSO-d$_6$): δC 126.98, 123.03, 126.11, 156.62, 160.67, 154.25, 134, 128, 131, 23.3.

LC-MS (70 eV): m/z=349(M+H+, 100%), 350(M+1, 19.5%), It indicates molecule contain 18 Carbon atoms (Figure 18).

5-(thiophen-2-yl)-3-p-tolylthieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine(8b)

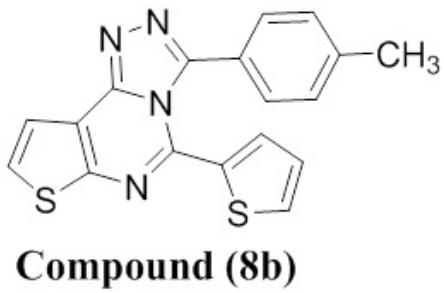


Figure 18: 5-(thiophen-2-yl)-3-p-tolylthieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 54% (Fine yellow crystals); m.p. 269-270°C.

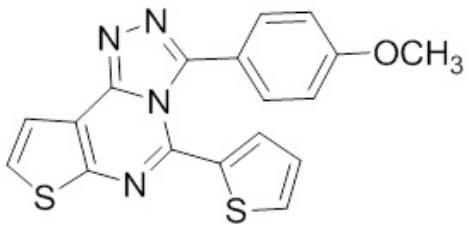
IR (KBr, cm^{-1}): 1155(-C-O-C Stretching), 3095(Ar C-H), 1680 (Ar C=C Stretching), 2950(SP_3 C-H Stretching).

^{1}H NMR (400 MHz; DMSO-d6): δH 6.96 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.3(1H,d, J_{HH}=7.0 Hz, Ar-H), 7.95(2H, d, J_{HH}=7.8 Hz, Ar-H), 7.03((2H, d, J_{HH}=7.8 Hz, Ar-H), 3.9(3H,S).

^{13}C NMR (100 MHz; DMSO-d6): δC 126.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131.

LC-MS (70 eV): m/z=365(M+H, 100%hpuihuiuio), 366(M+1,19.5), 3:1 It indicates molecule contain 18 Carbon atoms (Figure 19).

3-(4-methoxyphenyl)-5-(thiophen-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine(8c)



Compound (8c)

Figure 19: 3-(4-methoxyphenyl)-5-(thiophen-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 55% (yellow crystals); m.p. 234-235°C.

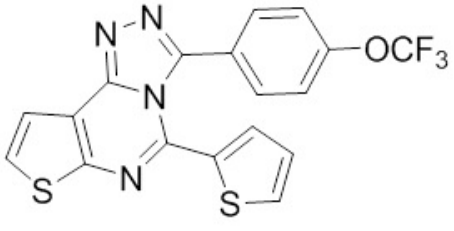
IR (KBr, cm^{-1}): 1140(C-O-C Stretching), 1340(C-F), 3110(Ar C-H), 1680 (Ar C=C Stretching).

^{1}H NMR (400 MHz; DMSO-d$_6$): δH 6.96 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.3(1H, d, J_{HH}=7.0 Hz, Ar-H), 7.95(2H, d, J_{HH}=7.8 Hz, Ar-H), 7.03((2H, d, J_{HH}=7.8 Hz, Ar-H), 7.84(1H,d), 7.22(1H,t), 8.33(1H,d).

^{13}C NMR (100 MHz; DMSO-d$_6$): δC 126.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131.

LC-MS (70 eV): m/z=365(M+H, 100%), 366(M+1, 19.5%), It indicates molecule contain 18 Carbon atoms (Figure 20).

5-(thiophen-2-yl)-3-(4-(trifluoromethoxy)phenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (8d)



Compound (8d)

Figure 20: 5-(thiophen-2-yl)-3-(4-(trifluoromethoxy)phenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 51% ((yellow crystals); m.p. 236-238°C.

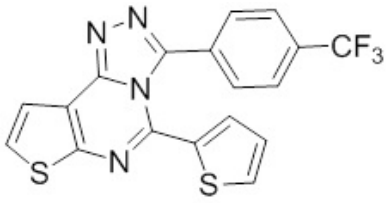
IR (KBr, cm^{-1}): 685 (-C-S-C), 1360(C-F), 3110(Ar C-H), 1687 (Ar C=C Stretching).

^{1}H NMR (400 MHz; DMSO-d$_6$): δH 6.96 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.3(1H,d, J_{HH}=7.0 Hz, Ar-H), 8.65(2H, d, J_{HH}=7.8 Hz, Ar-H), 7.73(2H, d, J_{HH}=7.8 Hz, Ar-H), 7.8(1H,d), 7.2(1H,t), 8.3(1H,d).

^{13}C NMR (100 MHz; DMSO-d$_6$): δC 126.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131, 124.

LC-MS (70 eV): m/z=403(M+H, 100%)+, 404(M+1, 19.5%), It indicates molecule contain 18 Carbon atoms (Figure 21).

5-(thiophen-2-yl)-3-(4-(trifluoromethyl)phenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (8e)



Compound (8e)

Figure 21: 5-(thiophen-2-yl)-3-(4-(trifluoromethyl)phenyl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 51% (Light brown crystals); m.p. 247-248°C;

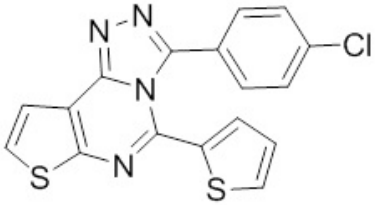
IR (KBr, cm^{-1}): 748(-C-Cl), 3120(Ar C-H), 1690 (Ar C=C Stretching).

^{1}H NMR (400 MHz; DMSO-d$_6$): δH 6.96 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.3(1H,d, J_{HH}=7.0 Hz, Ar-H), 8.25(2H, d, J_{HH}=7.8 Hz, Ar-H), 7.53((2H, d, J_{HH}=7.8 Hz, Ar-H), 7.8(1H,d), 7.2(1H,t), 8.3(1H,d).

^{13}C NMR (100 MHz; DMSO-d$_6$): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131, 124.

LC-MS (70 eV): m/z=369(M+H)$^+$, 371(M+2), 3:1 It indicates molecule contain one chlorine atom (Figure 22).

3-(4-chlorophenyl)-5-(thiophen-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine (8f):



Compound (8f)

Figure 22: 3-(4-chlorophenyl)-5-(thiophen-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 50% (Yellow powder); m.p. 268-270°C.

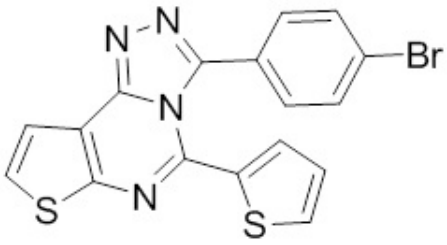
IR (KBr, cm^{-1}): 680(-C-S-C Stretching), 540(C-Br), 3120(Ar C-H), 1690 (Ar C=C Stretching).

^{1}H NMR (400 MHz; DMSO-d$_6$): δH 6.96 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.3(1H,d, J_{HH}=7.0 Hz, Ar-H), 7.65(2H, d, J_{HH}=7.8 Hz, Ar-H), 7.53(2H, d, J_{HH}=7.8 Hz, Ar-H), 7.83(1H,d), 7.26(1H,t), 8.36(1H,d).

^{13}C NMR (100 MHz; DMSO-d$_6$): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 128, 131, 124.

LC-MS (70 eV): m/z=413(M+H)$^+$, 414(M+2, 1:1 It indicates molecule contain one bromine atom (Figure 23).

3-(4-bromophenyl)-5-(thiophen-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine(8g)



Compound (8g)

Figure 23: 3-(4-bromophenyl)-5-(thiophen-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 48% (Yellow crystals); m.p. 193-195°C.

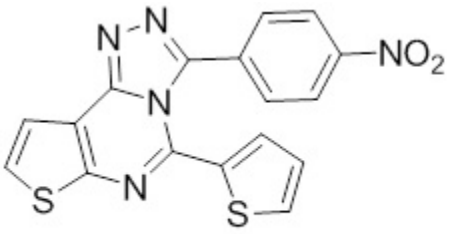
IR (KBr, cm^{-1}): 685(-C-S-C Stretching), 1540 and 1350 (N-O Stretching in Nitro group), 3160(Ar C-H), 1590 (Ar C=C Stretching).

^{1}H NMR (400 MHz; DMSO-d6): δH 6.76 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.2(1H, d, J_{HH}=7.0 Hz, Ar-H), 8.15(2H, d, J_{HH}=7.0 Hz, Ar-H), 8.53((2H, d, J_{HH}=7.0 Hz, Ar-H), 7.8(1H,d), 7.2(1H,t), 8.3(1H,d).

^{13}C NMR (100 MHz; DMSO-d6): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 144, 128, 124, 150.

LC-MS (70 eV): m/z=378(M-H, 100%)+, 379(M+1, 18.4%) It indicates molecule contain 17 carbon atoms (Figure 24).

3-(4-nitrophenyl)-5-(thiophen-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine(8h):



Compound (8h)

Figure 24: 3-(4-nitrophenyl)-5-(thiophen-2-yl)thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 70% (Yellow crystals); m.p. 279-281°C.

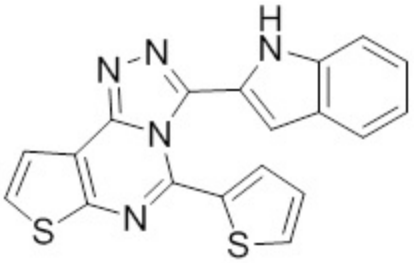
IR (KBr, cm^{-1}): 3132 (N-H Stretching), 3120(Ar C-H), 1680 (Ar C=C Stretching).

^{1}H NMR (400 MHz; DMSO-d$_6$): δH 6.86 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.2(1H, d, J_{HH}=7.0 Hz, Ar-H), 6.8(1H,S), 6.9(2H, m, Ar-H), 7.53-7.6(2H, m, Ar-H), 7.8(1H,d), 7.23(1H,t), 8.35(1H,d).

^{13}C NMR (100 MHz; DMSO-d$_6$): δC 127.92, 123.03, 126.11, 153.62, 160.67, 143.25, 124, 100, 128, 120, 113.

LC-MS (70 eV): m/z=374(M+H)$^+$, 375(M+1) It indicates molecule contain 19 carbon atoms (Figure 25).

3-(1H-indol-2-yl)-5-(thiophen-2-yl) thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine(8i)



Compound (8i)

Figure 25: 3-(1H-indol-2-yl)-5-(thiophen-2-yl) thieno[3,2-e][1,2,4]triazolo[4,3-c]Pyrimidine.

Yield: 60% ((Light yellow powder); m.p. 205-207°C.

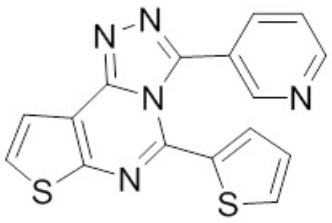
IR (KBr, cm^{-1}): 3110(Ar C-H), 1680 (Ar C=C Stretching).

^{1}H NMR (400 MHz; DMSO-d$_6$): δH 6.86 (1H, d, J_{HH}=7.0 Hz, Ar-H), 7.2(1H,d, J_{HH}=7.0 Hz, Ar-H), 8.5(1H,d, J_{HH}=7.4 Hz, Py. Ar-H), 7.6(1H, t, J_{HH}=7.4 Hz, Py. Ar-H), 8.7(1H, d, J_{HH}=7.4 Hz, Py. Ar-H), 9.3(1H, S, Py.Ar-H), 7.84(1H,d), 7.2(1H,t), 8.33(1H,d).

^{13}C NMR (100 MHz; CDCl$_3$): δC 127.92, 123.03, 126.11, 153.62, 160.67, 154.25, 134, 135, 124, 148, 155.

LC-MS (70 eV): m/z=334(M-H)$^+$, 335(M+1) It indicates molecule contain 16 carbon atoms (Figure 26).

3-(pyridin-3-yl)-5-(thiophen-2-yl) thieno[3,2-e] [1,2,4] triazolo[4,3-c] Pyrimidine (8j)



Compound (8j)

Figure 26: 3-(pyridin-3-yl)-5-(thiophen-2-yl) thieno[3,2-e] [1,2,4] triazolo[4,3-c] Pyrimidine.

Biological Activity

Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli* (clinical isolate) bacterial strains by disc diffusion method. A standard inoculum (1-2 × 10^7 cfu/ml 0.5 McFarland standards) were introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The disks measuring 6 mm in diameters were prepared from Whatman No. 1 filter paper and sterilized by dry heat at 140°C for 1 h. The sterile disks previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. Amoxicillin (30 µg) was used as positive control and the disk poured in DMSO was used as negative control and the test compounds were dissolved in DMSO at concentration of 100 and 50 µg/mL. The plates were inverted and incubated for 24 h at 37°C. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition of zone of measured and compared with controls. The bacterial zone of inhibition values is given in Table 1. The order of activity was 8i>8j>8e>8h>8d>8f >8g>>8a>8b>8c.

Zone of inhibition measure in mm								
Synthesized Compounds	**Gram positive**				**Gram negative**			
	Bacillus subtilis		*Staphylococcus aureus*		*Klebsiella pneumonia*		*Escherichia coli*	
	100 µg/mL	**50 µg/mL**	**100 µg/mL**	**50 µg/mL**	**100 µg/mL**	**50 µg/mL**	**100 µg/mL**	**50 µg/mL**
8a	7.5	3.5	8	7				
8b	7	4.5	7	4.5				
8c	6	3	7.5	5				
8d	10	8	11.1	9.5				
8e	11.5	9	12.5	11				
8f	9.5	7	9.5	7.5				
8g	8.5	6.5	9	6.5				
8h	11	9.5	11.5	8.5				
8i	13	10.5	15	11.5				
8j	12.5	10	14.5	10.5				
Amoxicillin								
Control (DMSO)								

Table 1: Anti-bacterial activity of compounds 8(a-j).

Antifungal studies

The newly prepared compounds were screened for their antifungal activity against *Candida albicans* and *Aspergillus flavus* in DMSO by agar diffusion method. Sabourauds agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH 5.7. Normal saline was used to make suspension of corresponding species. Twenty millilitres of agar media was poured into each Petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37°C for 1 h using an agar punch, wells were made and each well was labelled. A control was also prepared in triplicate and maintained at 37°C for 3-4

days. The fungal activity of each compound was compared with Ketoconazole as a standard drug. Inhibition zone were measured and compared with the controls. The fungal zone of inhibition values is given in Table 2.

Zone of inhibition measure in mm				
Synthesized Compounds	*Candida albicans*		*Candida albicans*	
	100 µg/mL	**50 µg/mL**	**100 µg/mL**	**50 µg/mL**
8a	8.5	5	7.5	5.5
8b	8	5.5	7	3.5
8c	6.5	4.5	7	4
8d	11.5	6.5	9	6
8e	13	11.5	10.5	8
8f	11	9	10	9
8g	9.5	7.5	8	6.5
8h	12.5	8	10.5	10
8i	17.5	12.5	16	12
8j	14.5	12	12.5	9.5
Ketoconazole	21	16	18.5	14
Control (DMSO)	---	---	---	---

Table 2: Anti-fungal activity of compounds 8(a-j).

Results and Discussion

Chemistry

The reaction sequences employed for synthesis of title compounds are shown in Figure 2. In the present work, the starting thieno[2,3-d]pyrimidine-2,4-diol(2) was prepared from methyl 2-amino thiophene-3-carboxylate (1) and Urea was prepared according to synthetic procedure [27]. 2,4-dichlorothieno[2,3-d]pyrimidine (3) was prepared according to synthetic procedure [28]. The 2-chloro-4-hydrazinylthieno[2,3-d] pyrimidine (4) was prepared according to synthetic procedure [29], which on further treatment with different Substituted Carboxylic acids 5(a-j) in $POCl_3$ gave 2-(5-chlorothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidin-3-yl)-5-Substituted benzene-1-ylium (6 a-j) according to synthetic procedure [30], which were treated with thiophene-2-boronic acid (7) under Suzuki reaction conditions to get Target Novel Thieno Pyrimidine derivatives (8a-j) according to synthetic procedure [31]. All compounds displayed IR, ^1H and ^{13}C NMR and mass spectra consistent with the assigned structures. ^1H NMR and IR spectrum of compounds (6 a-j) showed singlet at 2.3 ppm, 3.8 ppm are due to the aromatic methyl group protons and Aromatic methoxy group protons. The most characteristic IR absorption bands are at 1140 cm^{-1} (C-O-C), 740 cm^{-1} (C-Cl) and 1324 and 1552 cm^{-1} (N-O Stretching in Nitro group). The mass spectra of all the final derivatives showed comparable molecular ion peak with respect to molecular formula.

Anti-microbial studies

The newly synthesized compounds (8a-j) were screened for their *in-vitro* anti-bacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli* using Amoxicillin as standard by disc diffusion method (zone of inhibition) [32]. The test compounds were dissolved in di methyl sulfoxide (DMSO) at concentrations of 50 and 100 µg/mL. The antibacterial screening revealed that all the tested compounds showed good inhibition against various tested microbial strains compared to the standard drug. Along with the synthesized compounds 8i, 8j, 8e were found to be more active against tested bacterial strains as compared to the standard. Compound 8f exhibited moderate antibacterial activity against all tested bacterial stains. In general, increase of electron donating strength on the 1,2,4-triazole and Thiophene (methyl substitution) decreases antibacterial activity. On the other hand, introducing Electron withdrawing phenyl ring on the 1,2,4-triazole with thieno pyrimidine increases the antibacterial activity. The activity exhibited by the synthesized compounds were due to both 1,2,4-triazole and Thiophene core rings (Figure 2). The *in-vitro* antifungal activities for compounds 8a-8j were determined by agar diffusion method [32]. The results indicate that, among the tested compounds 8i and 8j were active against all tested fungal strains. The enhanced activities are due to electron withdrawing groups viz., $-CF_3$ and nitro attached to heterocyclic moieties (1,2,4-triazole and Thiophene) of thieno pyrimidine ring. All other compounds such as, 1, 2,4-triazole and Thiophene with methyl and methoxy groups in phenyl ring substitution with thieno pyrimidine showed lesser antifungal activity as compared with standard Ketoconazole. The Tables 1 and 2 depict the antimicrobial screening results of the final compounds.

Conclusion

The research study reports the successful synthesis and anti-microbial activity of 1, 2,4-triazole and Thiophene having thieno pyrimidine moiety. The anti-microbial activity study revealed that all the tested compounds showed good antibacterial and antifungal activities against pathogenic strains. The structure and biological activity relationship of title compounds indicate that the presence of electron withdrawing groups like $–CF_3$ groups attached to the triazole ring and Indole, Iso nicotinic ring and Thiophene rings were responsible for good antimicrobial activity and hence compounds 8i, 8j, 8e Exhibited more potent anti-microbial activity of all tested pathogenic strains.

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